
Anti-inflammatory and anti-bacterial activities of *Glycyrrhiza glabra* L.

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Glycyrrhiza glabra L. (family-Fabaceae-Leguminosae) is one of the important medicinal plant, commonly called as 'liquorice'. The main ingredient of liquorice is 'glycyrrhizin'. The present experiment was focused on the anti-inflammatory activity of hydro alcoholic extract of *G. glabra* (HAEGG) root against carrageenan induced rat paw oedema at dose levels of 100, 200, and 300 mg/kg orally. The HAEGG showed a maximum of 46.86% inhibitory action on carrageenan induced paw oedema at the dose of 200 mg/kg and inhibited the leukocyte migration in a dose dependent manner. The anti-inflammatory activity was comparable to the standard non-steroidal anti-inflammatory drug indomethacin (10mg/kg). The anti-bacterial activities of the methanol, ethyl acetate, acetone and chloroform extracts of *G. glabra* plant roots were tested against six bacterial species viz., *Bacillus coagulans*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhimurium* by the agar disc diffusion method. The results indicated that the extract of *G. glabra* showed various antibacterial activities (9-14mm/20µl inhibition zone) against the bacterial organisms tested. The methanol, ethyl acetate, acetone and chloroform extracts did not inhibit *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* but showed the inhibition effect against *B. coagulans*, *E. coli* and *S. typhimurium*. *Glycyrrhiza glabra* has significant anti-inflammatory and anti-bacterial activity with potential constituents targeting different components of inflammatory and anti-bacterial processes.

Key words: *Glycyrrhiza glabra*, Glycyrrhizin, peritonitis, prostaglandin, carrageenan, anti-bacterial

Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value to these plants lies in some chemical substances that produce a definite physiological action on the human body. The

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most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenol compounds (Hill, 1952). It is well known that infectious diseases account for high proportion of healthy problems, especially in the developing countries. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis, 1994). This resistance has increased due to indiscriminated use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists to search for new anti-microbial and anti-inflammatory substances from various sources, such as medicinal plants (Karaman *et al.*, 2003).

Secondary metabolites produced by plants constitute a source of bioactive substances and now-a-days the scientific interest has increased due to the search for new drugs from plant origin. *Glycyrrhiza glabra* is one of the important medicinal plants, commonly called as 'Liquorice'. This plant species are reported in the literature for its biological activities such as: anti-inflammatory and expectorant, controls coughing and has hormonal effects. It detoxifies and protects the liver. Medicinally, it is used internally for Addison's disease, Asthma, Bronchitis, Peptic ulcer, Arthritis, Allergic complaints and steroid therapy. Externally, liquorices are used for Eczema, Herpes and Shingles. Economically, the roots are boiled to extract the familiar black substance used in liquorice confectionery and this is sold dried to eat. Liquorice is also the basis for most proprietary laxatives and its extracts flavors beer, soft drinks and pharmaceutical products, and is used as a foaming agent in beers and fire extinguishers (Bown, 1995). The main ingredient of *G. glabra* is 'glycyrrhizin', a substance 50 times sweeter than sucrose, with cortisone-like effects. The present study was focused on the anti-inflammatory effects of the hydro alcoholic extract of *G. glabra* (HAEGG) root against carrageenan induced rat paw oedema and the anti-bacterial activities of methanol, ethyl acetate, acetone and chloroform extracts of the *G. glabra* root was tested invitro against six bacterial species by using the agar disc diffusion method. The bio-active marker 'glycyrrhizin' has been quantified using HPTLC.

Materials and methods

1000 g of air dried roots of *G. glabra* was extracted by cold maceration process with 70% hydro alcohol (Ethanol: water; 79:30) for 72 h. The hydro alcoholic extract (HAEGG) was rotary evaporated at 50°C and concentrated in vacuum to obtain a black- brown solid mass with a yield of 12.9% w/w. Phytochemical screening of the extract revealed the presence of quinones, flavonoids, alkaloids, terpenoids and amino acids (Kokate, 1994).

Acute oral toxicity of the HAEGG was studied in Swiss albino mice weighing 20-25g. HAEGG was administered orally at the dose of 100, 500, 1000, 1500 and 2000 mg/kg to six groups of animals (8 mice in each). Control group received normal saline (5ml/kg orally or 0.1 to 0.125 ml for mice weighing 20-25 g). Signs of toxicity and mortality within 24-72 h. were noted.

To determine the content of glycyrrhizin in the HAEGG, 500mg of HAEGG is made up to 25 ml with chloroform as quinones are reported to be easily soluble in chloroform (Zhong *et al.*, 1984). The resulting solution is centrifuged at 3000 rpm for 15 minutes and the supernatant was analyzed for glycyrrhizin content. 40 μ l of the filtered solution is applied on a 10x10 cm preactivated HPTLC silica gel 60 F 254 plate. HAEGG and standard glycyrrhizin were applied to the plate as 6 mm wide band with an automatic TLC applicator Linemat V with N₂ flow, 8 mm from the bottom. Densitometric scanning was performed on CAMAG scanner III at 265 nm. The plates were pre-washed by methanol and activated at 60°C for 5 min. prior to chromatography. The slit dimension was kept at 5 mm x 0.45 mm and 40mm/s scanning speed was employed. The mobile phase consisted of toluene: ethyl acetate: methanol (8:1:1) and 10 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 10cm x 10cm twin glass chamber saturated with the mobile phase. The analysis is repeated for six times and the possibility of interference from other components of HAEGG in the analysis was studied.

Carrageenan induced rat hind paw oedema model was used for anti-inflammatory screening (Winter *et al.*, 1962). Male Wister rats weighing 100-200 g were selected and provided feed and water ad libitum. Rats were fasted overnight before experiment was carried out. Fasted rats were divided into five different groups (n-6) of six animals each. Rats in Group I were given normal saline and were treated as control. Rats in Group II were administered indomethacin in normal saline at the dose of 10 mg/kg b.w. orally and were kept as standard (Hess and Milonig, 1972). Rats in Group III to Group V were administered orally with the HAEGG in normal saline at the doses of 100, 200 and 300 mg/kg b.w. respectively. The standard and HAEGG were given orally to the animals one hour prior to Carrageenan injection. Acute paw oedema was induced by injecting 0.1 ml of 1% (w/v) Carrageenan solution, prepared in normal saline in sub-plantar region of the left hind paw of the rat. The perimeter of paw was measured by using vernier caliper. Measurements were taken at 0,1,2,3 and 4 h. after the administration of the Carrageenan. Percentage protection or inhibition of oedema was calculated using the formula:

% inhibition of paw oedema= 100x (1- increase in paw volume of treated group/ increase in paw volume of control group).

Inflammation was induced by the modified method of Griswold *et al.* (1987). Male Swiss albino mice weighing 20-25 g were divided into five groups (n=4). Group I served as control. Group II served as standard and was dosed with indomethacin (10 mg/kg. p.o) and Groups III-V were dosed with HAEGG at the doses of 100, 200, 300 mg/kg. p.o. The standard drug and HAEGG were administered orally one hour prior to the induction of peritonitis. After one hour, Carrageenan (0.25 ml, 0.75% w/v in saline) was injected intraperitoneally. Four hours later, the animals were sacrificed by cervical dislocation and 2 ml of Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) was injected into the peritoneal cavity. Following a gentle massage, peritoneal exudates were removed. The total leukocyte count was determined in a Neubauer chamber and the differential cell count was determined (D' Amour *et al.*, 1965).

The percentage of the leukocyte inhibition was calculated using the following formula:

$$\text{Leukocyte inhibition (LI \%)} = (1-T/C) \times 100$$

Where 'T' represents the treated groups leukocyte count and 'C' represents the control group leukocyte count.

Inhibition of Neutrophil migration was calculated using the following equation:

$$\text{Inhibition of Neutrophil migration} = 100([NT/NC] \times 100)$$

Where, NT= Neutrophil counts of treated groups; NC= Neutrophil counts of control groups.

Roots of liquorice were taken, dried and used in this study to determine its anti-bacterial activity against the six bacterial species such as *Bacillus coagulans*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhimurium*. The liquorice roots were dried and broken into small pieces under sterile conditions and 20 g of plant root part was extracted with 150 ml of ethyl acetate, methanol, chloroform and acetone solvent (Merk, Darmstadt) for 24 h. by using Soxhlet apparatus (Bradshaw, 1992). All the extracts thus obtained were injected into empty sterilized antibiotic discs of 6 mm diameter in amounts of 20 μ l. Discs injected with 20 μ l of pure ethyl acetate, methanol, chloroform and acetone served as negative controls.

All the bacteria mentioned above were incubated at $30 \pm 0.1^\circ\text{C}$ for 24 h. by inoculation into nutrient broth (Difco). Sterilized petridishes (9 cm diameter) were inoculated with 0.01 ml of one of the above culture media (10^5 - 10^6 bacteria per ml). Muller- Hinton agar (oxid) sterilized in a flask and cooled to 45 - 50°C was distributed by pipette (15 ml) into each inoculated petridish and swirled to distribute the medium homogenously. Discs injected with extracts were applied on the solid agar medium by pressing slightly. The treated petridishes were placed at 4°C for 1-2 h. and then incubated at $35 \pm 0.1^\circ\text{C}$ for 18-24 h. (McCuen and McCuen, 1988; Jones *et al.*, 1989). At the end of the period, the inhibition zones formed on the media were measured with a transparent ruler in millimeters.

Results are expressed as mean \pm S.D. and difference in means are determined by one-way ANOVA followed by post -hoc analysis with Dunnet's t-test; values $P < 0.05$ were considered as statistically significant.

Results and discussion

Glycyrrhiza glabra is one of the important medicinal plants. The whole plant and its root mainly have been used as a folk medicine in many countries, for the treatment of Rheumatic pain, Addison's disease, Asthma, Bronchitis, Peptic ulcer, Arthritis, and allergic complaints. Despite the traditional use of the species in rheumatic diseases, the anti-inflammatory and anti-bacterial activity of the *G. glabra* has not been studied in detail in the past.

Inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents. The development of carrageenan induced inflammation is a biphasic event, the first phase occurs within an hour of injection is attributed to the release of histamine, 5-HT and kinins, while the second phase which can be measure around 3-4 h. time is related to the release of prostaglandins (Vane and Botting, 1998; Agarwal, 2006). The presence of prostaglandin E_2 in inflammatory exudates from the injected foot can be demonstrated at three hours time period and thereafter (Vinegar *et al.*, 1987). Indomethacin is used as standard reference drug as it is reported to inhibit inflammation by its effect upon plasma exudation associated with carrageenan mediated inflammation (Agarwal, 2006; Maria and Sergio, 1978). HAEGG showed a maximum of 46.86% oedema inhibition at 3 h. at the dose of 200 mg/kg and the effect tested for 3 h. for *G. glabra* (Table 1). The inhibitory activity decreased after 3 h. and above 200 mg/kg dose, the anti-inflammatory effect recedes.

Intra-peritoneal injection of carrageenan leads to inflammation of the peritoneum resulting from carrageenan induced release of interleukin-1 from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory

cytokine, induces accumulation of polymorpho nuclear cells by a variety of processes including adhesion and cell mobility (Meade *et al.*, 1986). Leukocyte aggregation is a fundamental event during inflammation. Cell migration occurs as a result of such different process including adhesion and cell mobility.

In the present study the standard NSAID, indomethacin (10 mg/kg) has produced 63.34% of leukocyte inhibition and 43.14% inhibition of neutrophil migration. HAEGG exhibited a maximum of 48.83% leukocyte inhibition at 300 mg/kg dose (Table 2) and the effect increases dose dependently. The results suggest that the HAEGG have potential constituents interacting with the different cellular processes of inflammation. As, glycyrrhizin is reported to have cytotoxic activity, its quantity in the HAEGG is of greatest importance in terms of toxicity and biological activity. Hence, we quantified the amount of glycyrrhizin in the hydro alcoholic extract and studied the acute toxicity in albino mice. The spot at $R_f=0.68$ corresponding to glycyrrhizin was observed in the chromatogram of the HAEGG along with other components. There is no interference from other components present in the chromatogram. The HAEGG was found to contain 0.41% (w/w) of glycyrrhizin in dried extract.

The mice administered with the HAEGG showed no toxic signs or mortality up to a dose of 2000 mg/kg showing the safety of the extract. Glycyrrhizin which is also found as active ingredient in the species of *G. glabra*, reported to have significant anti-bacterial and anti-oxidant activity (Ayfer and Ozlem, 2003) against some strains of bacterial species. The observed anti-inflammatory activity of HAEGG can also be attributed to the anti-oxidant potential of the glycyrrhizin in the extract, as inflammation involves oxidative damage.

The invitro anti-bacterial activities of the dried root extracts of *G. glabra* are shown in the Table 3. In addition, the inhibition zones formed by standard antibiotic discs and those discs injected with only ethyl acetate, methanol, chloroform and acetone (negative controls), are also listed in Table 3. *G. glabra* root extracts showed various antibacterial activities (9-14 mm/20 μ l inhibition zone) against the bacterial organisms' viz., *Bacillus coagulans*, *Escherichia coli*, and *Salmonella typhimurium* were tested. The alcohol, ethyl acetate, acetone and chloroform extracts did not inhibit *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

As can be seen from Table 3, *B. coagulans*, *E. faecalis*, and *P. aeruginosa* were resistant to Cefedizime (30 μ g), Oxalin (1 μ g) and Ampicillin (10 μ g). The methanol and chloroform extracts of *G. glabra* showed the maximum inhibition effect against *E. coli*, *B. coagulans* and *S. typhimurium*. This study indicated that methanol and chloroform extracts of the root of *G. glabra* showed potential antibacterial activity against the tested organisms.

As shown in Table 3, the control discs injected with 20 μ l of methanol, chloroform, ethyl acetate and acetone showed no inhibitory effect against the microorganisms tested. It is not surprising that there are differences in the anti-bacterial effects of plant groups, due to the phytochemical differences between species (Ayfer and Ozlem, 2003; Srinivasan *et al.*, 2006). The present study supports the view that *G. glabra* root extracts might be useful as anti-bacterial agents. Based on ethanopharmaceutical data novel drugs may be developed following series of experiments to search for anti-inflammatory and anti-bacterial agents (Agarwal, 2006; Ayfer and, 2003; Charles Dorni, 2006).

Table 1. Effect of *G. glabra* and carrageenan- induced paw oedema in rats (n=6).

Groups	Paw volume (cm mean \pm SD)				Oedema value (cm mean \pm SD)			
	1 h	2h	3h	4h	1h	2h	3h	4h
Control	0.62 \pm 0.02	0.67 \pm 0.24	0.74 \pm 0.04	0.69 \pm 0.02	0.21 \pm 0.01	0.24 \pm 0.02	0.31 \pm 0.02	0.26 \pm 0.02
Indomethacin (10 mg/kg)	0.56* \pm 0.02	0.57* \pm 0.02	0.55* \pm 0.02	0.53* \pm 0.02	0.16* \pm 0.01 (23.82)	0.17* \pm 0.02 (32.0)	0.15* \pm 0.02 (53.14)	0.13* \pm 0.01 (51.95)
GG (100 mg/kg)	0.58 \pm 0.02	0.62* \pm 0.02	0.66* \pm 0.02	0.63* \pm 0.02	0.18 \pm 0.02 (14.28)	0.21* \pm 0.03 (16.05)	0.24* \pm 0.04 (21.84)	0.22 \pm 0.03 (18.54)
GG (200 mg/kg)	0.56* \pm 0.02	0.58* \pm 0.02	0.56* \pm 0.02	0.58* \pm 0.03	0.17 \pm 0.01 (19.06)	0.19* \pm 0.02 (24.2)	0.17* \pm 0.01 (46.86)	0.19* \pm 0.02 (29.62)
GG (300 mg/kg)	0.56* \pm 0.02	0.58* \pm 0.02	0.61* \pm 0.01	0.60* \pm 0.02	0.16* \pm 0.02	0.18* \pm 0.02	0.21* \pm 0.03	0.20* \pm 0.03

Values represent the mean \pm SD of 6 animals of each group;

Values in parenthesis indicate the percentage inhibition rate.

* Experimental groups compared with control (p<0.05) statistically significant.

Table 2. Effect of *G. glabra* on carrageenan induced leukocyte aggregation (n=4).

Groups	Total leukocyte count 10 ⁶ cells/cm	% Leukocyte inhibition	Neutrophils (10 ⁵ m L ⁻¹)	% inhibition of Neutrophil migration
Control	16.12 \pm 2.2	-	0.68 \pm 0.04	-
Indomethacin(10mg/kg)	5.94 \pm 2.15*	63.34	0.38 \pm 0.02	43.14
GG 100 mg/kg	12.84 \pm 2.2	23.45	0.54 \pm 0.04*	22.68
GG 200 mg/kg	10.18 \pm 0.64*	36.82	0.52 \pm 0.04*	25.38
GG 200 mg/kg	8.26 \pm 2.4*	48.83	0.42 \pm 0.28*	41.32

Values are mean \pm SD, * Experimental groups were compared with control (p<0.05) statistically significant.

Table 3. The anti-bacterial activities of methanol, ethyl acetate, acetone and chloroform extracts of the dried extracts of *Glycyrrhiza glabra* and the inhibition zones formed by standard antibiotic discs and discs injected with only solvents (negative controls).

Microorganisms	Inhibition zone (mm/20µl)				Diameter of inhibition zone			
	<i>Glycyrrhiza glabra</i>				Standard antibiotics			
	Methanol A	Ethyl acetate B	Acetone C	Chloroform D	Amphicillin (10µg)	Cefidizime (30µg)	Oxallin (1µg)	Control A B C D
<i>Bacillus coagulans</i>	12	7	8	10	10	–	–	-----
<i>Enterococcus faecalis</i>	–	–	–	–	10	13	–	-----
<i>Escherichia coli</i>	14	9	11	10	20	–	–	-----
<i>Pseudomonas aeruginosa</i>	–	–	–	–	–	12	–	-----
<i>Salmonella typhimurium</i>	11	8	9	9	15	16	–	-----
<i>Staphylococcus aureus</i>	–	–	–	–	14	18	–	-----

Extraction solvent (20 µl) control

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